IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Shimon Sakaguchi
Serial No. : 09/284,114
Filed : April 7, 1999
Title : A MOUSE STRAIN WITH NATURAL ONSET OF AUTOIMMUNE ARTHRITIS

Art Unit : 1633
Examiner : E. Sorbello

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Shimon Sakaguchi, Ph.D., having an address at Higashi-hiraki-cho 20, Takano, Sakyo-ku, Kyoto 606-8107, Japan, am the sole inventor of the above-referenced United States patent application serial no. 09/284,114. I am a professor of Experimental Pathology at the Institute for Frontier Medical Sciences, Kyoto University, and am an expert in the general fields of experimental pathology and immunology, particularly, in the field of arthritis. I was considered an expert in these fields in 1996 at the time of the invention.

2. I have read the specification and the file history, including past and the outstanding office actions, and Applicant's responses, for the above-identified patent application. I understand the issues presented by the Patent Office in the Office Action mailed April 25, 2001, and the Advisory Action mailed November 23, 2001, regarding the pending claims of the application (referred to hereinafter as "the invention"). I further understand that a response was filed with a Request for Continued Examination on January 9, 2002 addressing all outstanding issues. I am submitting this declaration in further support of the arguments presented in that response.

3. As discussed in previous communications with the Patent Office, from the artificial breeding environment I created, I was able to develop a stable strain of mice that developed rheumatoid arthritis, which is deposited with the International Patent

Organism Depository National Institute of Advanced Industrial Sciences and Technology accession number FERM BP-7790.

4. It is the claimed understanding that one of the Patent Office's concerns is that the claimed claimed mouse strain does not possess identifiable characteristics such as biochemical markers and other phenotypic characteristics to distinguish it from the original BALB/c mouse strain, such as taught by U.S. Patent No. 6,040,495 and the mouse strain that it discloses.

5. There are, however, many identifiable characteristics of the claimed mouse strain to distinguish it from the original parent BALB/c mouse stain. First, normal BALB/c mice do not develop rheumatoid arthritis by their sixth month. Second, normal BALB/c mice do not develop rheumatoid arthritis having at least one of the characteristics described in the application. For example, normal BALB/c mice do not normally develop rheumatoid arthritis which pathohistologically tends to resemble human rheumatoid arthritis in its chronic progression from the appearance of pannus to the inflammatory destruction of joint cartilage and bone accompanied by lymphocyte infiltration. Clinically, normal BALB/c mice do not develop rheumatoid arthritis that tend to resemble human rheumatoid arthritis in that the small and large joints of the forelegs and hind legs are affected symmetrically, and the lesions chronically progress and finally lead to joint stiffening. Finally, normal BALB/c mice do not develop rheumatoid arthritis that tend to resemble human rheumatoid arthritis in that rheumatoid factor, autoantibody against type II collagen specific for joints, and hypergammaglobulinemia develop with high frequency.

6. Evidence of the differences between normal BALB/c mice and mice of the present invention can be found in the specification. For example, the differences can be seen by comparing Figures 1 and 3 of a mouse of the present invention with Figures 2 and 4 of a normal BALB/c mouse. Swelling of the joints of the forelegs and the hind legs show the mouse of the invention having developed arthritis. Figures 5 and 7 are X-ray photographs that show that the cartilage and bone are destroyed

symmetrically in the large and small joints of the legs, which can be compared to the X-ray photographs of a normal BALB/c mouse shown in Figures 6 and 8. Figures 9 and 11 show the microscopic photographs of a section of joint tissue of a mouse of the present invention, which can be compared to the microscopic photographs of a normal BALB/c mouse of shown in Figures 10 and 12. Figures 14, 15, and 16 show mice of the invention having significantly increased titres of rheumatoid factor, autoantibody against type II collagen, and serum IgG levels as compared to normal BALB/c mice.

7. In further support of the distinctiveness of the claimed mouse strain and those of the parent normal BALB/c mouse strain, I provide additional evidence in Figs. 1, 2, and 3 that distinguishes the claimed mouse strain from that of the normal BALB/c mouse strain. For example, Fig. 1 shows the staining of thymocytes from a 2-month-old SKG mouse (SKG mice are mice of the claimed mouse strain) and a BALB/c mouse by anti-CD3 antibody. The thymi of SKG mice showed decreased expression levels of CD3 on immature thymocytes and reduction of mature $CD3^{high}$ thymocytes. Moreover, Fig. 2 shows that in SKG mice, the number and percentage of T cells, both $CD4^+$ cells and $CD8^+$ cells in the spleen and lymph nodes, as well as CD4 or CD8 single-positive thymocytes, decreased by a third to two thirds as compared to age-matched BALB/c mice by two months of age. The results in Figs. 1 and 2, taken together, indicate that the genetic abnormality in SKG mice may be responsible for the altered differentiation of thymocytes, decreased thymic production of mature T cells, and resultant T-lymphocytopenia.

8. Further experimentation confirms that the cause of rheumatoid arthritis in the claimed mouse strain is due to a genetic mutation. To determine whether the cause of the arthritis was a genetically determined abnormality or vertical/horizontal transmission of arthriotenic microbes, we crossed SKG mice to normal BALB/c mice and monitored the offsprings for six months for the development of arthritis. The results are shown in Fig. 3. No arthritis developed in the offsprings from the crossings between SKG males and normal BALB/c females (c x skg) or between SKG females and normal BALB/c males (skg x c). By contrast, arthritis developed in approximately 50% of the

N2 generation obtained by crossing SKG males with the above non-arthritic F1 hybrids [(skg x c)F1 x skg]. The development of arthritis took a similar clinical pattern and showed similar disease severity as the arthritis of SKG mice. Taken together, these results indicate that the cause of the arthritis was not microbial infection but genetical abnormality, presumably of a single gene locus, and that the abnormality was inherited in an autosomal recessive fashion with nearly 100% penetrance of the trait in SKG homozygotes.

9. Based on the data obtained from numerous experiments, provided in the specification and supplemented here, the mouse strain claimed in the application is genetically homozygous and stable, with phenotypic differences that clearly distinguish it over the parent mouse strain.

10. I hereby declare that all statements made herein of the claimed own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully Submitted

Date: April 25, 2002

Shimon Sakaguchi

Shimon Sakaguchi, Ph.D.